INDIAN JOURNAL OF MICROBIOLOGY

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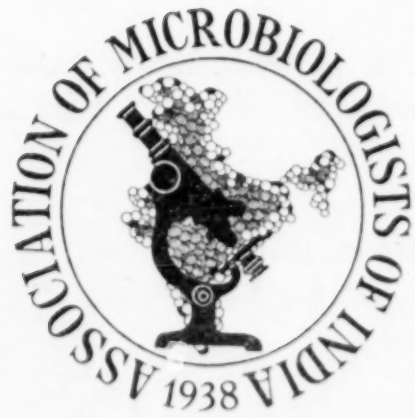
The Indian Journal of Microbiology will be published quarterly on the last day of March, June, September and December.

Members of the Association will receive the Journal free. For non-members, the annual subscription is Rs. 15/- (inland) and Rs. 20/- (overseas). Single issues are priced at Rs. 4/- (inland) and Rs. 6/- (overseas).

Volume 1 January—March, 1961 Number 1

INDIAN JOURNAL OF MICROBIOLOGY

Edited for the Association of Microbiologists of India



Published quarterly by the
ASSOCIATION OF MICROBIOLOGISTS OF INDIA
P207 PRINSEP STREET, CALCUTTA-13

Acknowledgment

The authors' sincere thanks are due to to Prof. B. C. Guha, Department of Applied Chemistry, Calcutta University, for his advice. The technical assistance of Sri B. Sanyamath and Sri D. Sengupta is also acknowledged.

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INDIAN
JOURNAL OF
MICROBIOLOGY

Vol. I, No. 1, 1961

OCCURRENCE OF A STRAIN OF TURNIP CRINKLE VIRUS IN INDIA

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From the Department of Botany, Lucknow University, Lucknow

(Received for publication, November 1959)

During 1958, turnip plants grown in two plots near Lucknow University were found affected by crinkle disease. The leaves presented rugged appearence, developed yellow patches and were brittle. The yellow patches coalesced and became necrotic with the age of the plants. In course of about a month, the affected leaves began to die and wither away. The growth of the diseased plants was much arrested and they were rosette in appearence.

Materials and Methods

Every g. of diseased leaves was crushed with 1 ml. of sterile distilled water and the extract was centrifuged for 15 min. at 3,000 r.p.m. Plants were inoculated with pale brown supernatent liquid and were kept in insect-proof conditions. Carborundum powder was used as an abrasive.

Results

The disease was successfully transmitted by means of mechanical inoculations but attempts to transmit it through aphids (*Myzus persicae* Sulz.) and cuscuta (*Cuscuta reflexa* L.) were not successful.

Host Range of the Virus

Turnip (*Brassica rapa* L.)

Disease symptoms appeared with yellow spots which soon coalesced to give chlorotic appearence to the leaves with necrosis. Later, the leaves crinkled and withered away. Leaves emerging after inoculation were diseased and the plant became rosette in appearence with stunted growth (Plate I, Fig. 1.)

Cauliflower (*B. oleracea* var. *botrytis* L.)

The most pronounced symptoms of the disease were curling and vein banding, mostly in young leaves and growth of the plants were arrested.

Cabbage (*B. oleracea* var. *capitata* L.)

Mottling with the appearance of minute chlorotic or necrotic lesions was observed. The growth of the plant was retarded after infection (Plate I, Fig. 2A).

Kohlrabi (*B. oleracea* L. var. *caulorapa* Pasq.)

This plant was highly susceptible. The leaves puckered or curled with blistering and necrosis. The growth of the plant was also retarded after infection (Plate I, Fig. 2B.)

The symptoms developing on plants, belonging to the families other than cruciferae, are summarized in Table I.

TABLE I.

Showing symptoms developing on plants

Family	Host Plant	Characteristic symptoms.
Solanaceae	*Nicotiana tabacum* L. var. *Turkish*	Inoculated leaves became slightly chlorotic with necrotic patches. Very young leaves emerging after infection remained thin and yellowish.
,,	*N. plumbaginifolia* L.	Slight chlorosis of leaves. New leaves emerging after infection remained narrow, fragile and yellowish.
,,	*N. glutinosa* L.	Leaves puckered and occasionally developed faint vein banding.
,,	*Lycopersicon esculentum* Mill.	Chlorosis immediately followed by necrosis; the whole compound leaf with rachis tended to curl downward. The disease was systemic.
,,	*Solanum tuberosum* L.	The symptoms resembled those on tomato with the difference that the whole leaf did not tend to curl downward. Necrosis was not severe.
,,	*S. nigrum* L.	Chlorosis followed by necrosis.
,,	*Datura stramonium* L.	Fairly visible light chlorotic lesions; the margins of leaves were slightly curled inward.
Leguminoseae	*Cymopsis tetragonoloba* (L) Taub.	Minute necrotic lesions appeared on the leaflets after inoculation, non inoculated halves remained normal.
Chenopodiaceae	*Beta vulgaris* L.	Sometimes slight curling and mottling of leaves was observed.

Necrotic local lesions were not observed on the inoculated leaves of *Gomphrena globosa* L. and *Chenopodium amaranticolor* Costs and Reyn.

Physical properties of virus

Dilution end point

The standard extract was diluted with sterile distilled water and six young vigorously growing turnip, cauliflower, kohlrabi and cabbage plants were inoculated with each dilution. The number of plants infected with various dilutions is given in Table II. Positive infections were obtained regularly upto the dilution of 10^{-5} but not at 10^{-6}.

Thermal inactivation point

This was determined by heating 2 ml. samples of standard extract on a water bath for 10 min. at various temperatures. The virus becomes inactivated between 85°C—90°C.

TABLE II.

Dilution end point of the virus in the extract

Dilution of the extract	Turnip	Cauli-flower	Kohlrabi	Cabbage
1/10	6	5	6	6
1/100	6	5	6	5
1/1,000	5	2	5	3
1/10,000	4	2	3	1
1/1,00,000	2	1	1	1
1/10,00,000	1	Nil	Nil	Nil

Longevity of the virus in vitro

The virus in the sap was infective for about 2 weeks at room temperature (25°C — 30°C).

Serological reaction

Antiserum of the virus reacted with antigen upto the dilution of 1/160 or more. 1/10 dilution of the antiserum gave visible coarse granular precipitate upto 10^{-5} dilution of antigen and this was the end point.

Discussion

Since Gardner and Kendrik (1921) reported "turnip mosaic", the first cruciferous virus disease, Clayton (1930), Hoggan and Johnson (1935), Tompkins and Thomas (1938), Walker *et al.* (1945), Sylvester (1953) and several others have studied various virus diseases of cruciferous plants. All of them were found to be transmissible by aphids. Markham and Smith (1949) described turnip yellow mosaic virus (TYMV), the first virus attacking crucifers, which was not transmitted by aphids but was transmitted by flee-beetles. The virus disease investigated here, although not transmissible by aphids, is not related to turnip yellow mosaic virus because of marked differences in host reactions, host range and resistance to heat. Broadbent and Heathcote (1957) have reported turnip

crinkle virus, which was also not transmissible by aphids. The virus described by us resembles this virus in certain properties i.e. host reactions, host range and dilution end point but seems to be a new strain because of the differences in thermal death point, transmissibility to *Nicotiana* spp. and failure to produce necrotic local lesions on *G. globosa* L. and *C. amaranticolor* Costs and Reyn.

Summary

A virus disease of turnip (*Brassica rapa* L.) has been described. It is characterised by crinkling, stunting and rosetting of plants and is readily transmissible by mechanical means but not by aphids. The virus has a dilution end point of 10^{-5} and thermal inactivation point between 85°C—90°C. It can infect cauliflower (*B. oleracea* L. var. *botrytis* L.), kohlrabi (*B. oleracea* L. var. *caulorapa* Pasq.), Cabbage (*B. oleracea* L. var. *capitata* L.), tobacco (*Nicotiana* spp.), tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), nightshade (*S. nigrum*. L.), datura (*Datura stramonium* L.), guar (*Cymopsis tetragonoloba* L. Taub.) and sugarbeet (*Beta vulgaris* L.). The virus appears to be a new strain of turnip crinkle virus.

Acknowledgment

The authors are grateful to Scientific Research Committee, U.P. for financial assistance.

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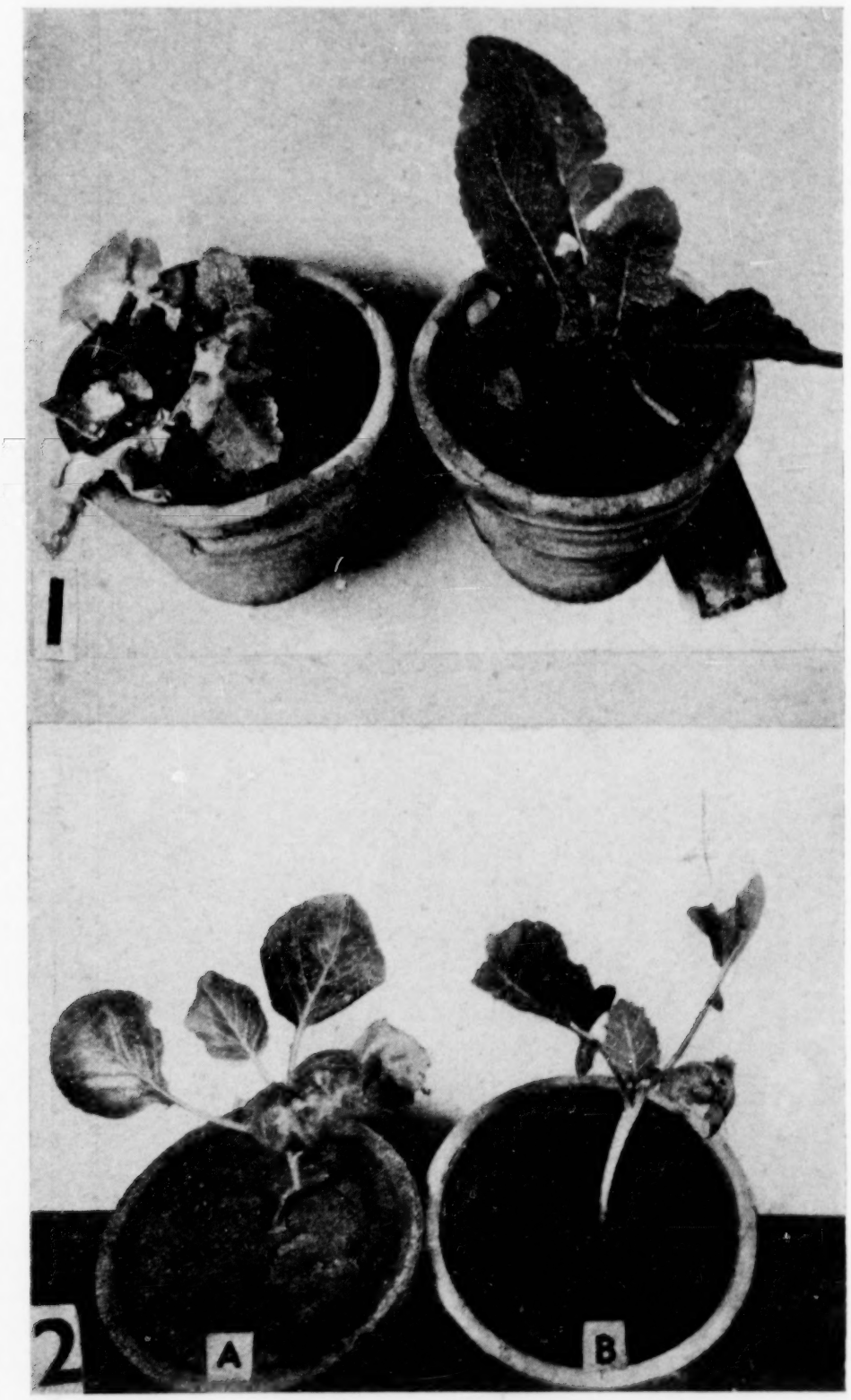
Explanation of Plates

Plate I

Diseased and healthy turnip plants. Yellowing and crinkling of leaves and stunted growth of diseased plant are clearly visible.



A. Diseased cabbage plant with minute chlorotic lesions. Some of the lesions have coalesced along the veins.

B. Diseased kohlrabi plant. Leaf lamina has curled and shrunken with blistering.

TAL STATI

3702

INDIAN
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Vol. I, No. 1, 1961

THE EFFECT OF POTASSIUM NUTRITION ON MULTIPLICATION OF TOBACCO MOSAIC VIRUS IN TURKISH TOBACCO (*NICOTIANA TABACUM* L.) PLANTS

G. S. VERMA AND J. P. VARMA

From the Department of Botany, Lucknow University, Lucknow

(Received for publication, November 1959)

Various workers have studied the effect of nitrogen, phosphorus and potassium and such minor elements as zinc, manganese and iron on virus multiplication in plants. The effect of nitrogen has been studied more intensively than that of potassium (Cheo *et al.*, 1952; Pound and Weathers, 1953; Weathers and Pound, 1954; Bawden and Kassanis, 1950; Spencer, 1939; 1941) on virus activity. The present paper deals with the effect of different levels of potassium on height, fresh and dry weights and virus concentration of the Turkish tobacco (*Nicotiana tabacum* L.) plants systemically infected with tobacco mosaic virus.

Materials and Methods

Six-inch clay pots were painted inside with bitumen paint, drainage holes plugged with glass wool and covered with watch glasses. They were filled with purified sand (Hewitt, 1946) and housed in insect-proof glass house. Arnon and Hoagland's (1940) nutrient solution was added to the sand and seeds of Turkish tobacco (*N. tabacum* L.) were sown. After 20 days the seedlings grew to a 3-4 leaf stage. Seedlings of uniform size were selected and each was transplanted in a separate pot. The pots were grouped into 4 batches, 30 pots in a batch, and were irrigated twice a week with Arnon and Hoagland's solution containing 3,900, 390, 39 and 0 ppm of potassium (KNO_3). Sufficient solution was added to allow free flow of drainage. The accumulation of salts was checked to a considerable extent by flushing the pots with distilled water.

Twenty days later, when potassium deficiency symptoms became clear in batch receiving 0 ppm of potassium, tobacco mosaic virus was inoculated into all the four batches of plants. Two or three lower leaves per plant were inoculated using carborundum powder as an abrasive. Control plants were not inoculated.

At 0, 15 and 30 days after inoculation, 4 plants from each batch of inoculated and 4 plants from the control were uprooted and their mean length in cm., fresh and dry weights in g. were determined. The dry weight, without the roots, was determined after heating the plants for 10 hr. at 100°C and then at 80°C till the weight was constant. Rest of the plants were homogenised with distilled water, in the proportion of 1 ml. of distilled water to one g. of wet plant tissue, in pestle and mortar. The juice was

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3702

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expressed through muslin cloth and stored in a deep freeze. For assay of virus concentration in the juice, counts of local lesions produced on half leaves of *N. glutinosa L.* were taken. The juice was heated to 60°C for 10 min. and centrifuged at 3,000 r.p.m. for 15 min. before inoculation on the leaves. For optical density readings by Unichem spectrophotometer (Takahashi, 1951) a quantity of the expressed sap was alternately centrifuged at low (3,500 r.p.m.) and high (12,000 r.p.m.) speed for 15 min. in angle centrifuge and the supernatant was diluted 1/100 in distilled water.

Total nitrogen content of the heat clarified sap was determined by the micro-kjeldahl method.

The experiment was conducted during the months of December and February. The temperature inside the glasshouse was in the range of 20°C — 30°C.

Results

Height, fresh and dry weights of the diseased and healthy plants supplied with different concentrations of potassium are presented in Tables I — III. When the concentration of potassium was increased or decreased beyond 390 ppm, adverse effects on height, fresh and dry weights of Turkish tobacco plants, both infected and uninfected, were observed (Tables I — III ; Plate I, Figs. 2 and 3). The fresh and dry weights of the infected plants fell considerably below normal on the 50th day. The height was not so much affected.

TABLE I.

Effect of potassium on the height of healthy and diseased plants of Turkish tobacco (N. tabacum *L.*)

Potassium	Interval (days)*					
	20		35		50	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
0 ppm	6·3	6·3	10·5	8·0	19·2	16·6
39 ppm	8·8	8·8	15·0	13·0	59·7	36·5
390 ppm	12·5	12·5	27·5	16·0	68·5	40·3
3900 ppm	10·5	10·5	23·5	14·0	49·3	36·4

* Time between transplantation and harvest of plants. The figures represent average height of 4 plants expressed in cm.

The most pronounced symptoms of potassium deficiency were interveinal chlorosis and scorching of margins and tips of older leaves (Plate I, Fig. 1). Tobacco mosaic virus infection in those plants was quite indistinct (Plate I, Fig. 3), the leaf size was not drastically reduced.

Increase beyond 390 ppm of potassium resulted in the decrease in the amount of active virus produced by the plants. Plants grown under potassium deficient conditions (0 ppm) produced very little virus (Table IV) and the growth was also stunted.

TABLE II.

Effect of potassium on fresh weight of healthy and diseased plants of Turkish tobacco (N. tabacum *L.*)

Potassium	Interval (days)*					
	20		35		50	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
0 ppm	0·49	0·49	1·31	0·80	6·98	6·78
39 ppm	1·17	1·17	9·94	3·62	43·14	12·78
390 ppm	2·65	2·65	16·93	13·40	100·78	16·88
3900 ppm	2·45	2·45	17·47	6·69	44·38	15·35

* Time between transplantation and harvest of plants. The figures represent average weight of 4 plants expressed in g.

TABLE III.

Effect of potassium on dry weight of healthy and diseased plants of Turkish tobacco (N. tabacum *L.*)

Potassium	Interval (days)*					
	20		35		50	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
0 ppm	0·04	0·04	0·16	0·10	0·82	0·77
39 ppm	0·11	0·11	0·91	0·41	4·62	1·42
390 ppm	0·19	0·19	1·28	1·29	10·22	1·92
3900 ppm	0·17	0·17	1·36	0·58	4·45	1·81

* Time between transplantation and harvest of plants. The figures represent the average weight of 4 plants expressed in g.

TABLE IV.

Effect of potassium on the active virus formation in diseased plants of Turkish tobacco (N. tabacum *L.*)

Potassium	Interval (days)*						
	20	35			50		
	—	Dilution			Dilution		
	—	1/10	1/100	1/1000	1/10	1/100	1/1000
0 ppm	—	6	3	2	8	3	2
39 ppm	—	315	138	61	733	411	149
390 ppm	—	295	112	52	741	465	172
3900 ppm	—	267	110	56	550	397	138

* Time between transplantation and harvest of plants. The figures represent local lesions produced by heat clarified sap inoculated on 30 half leaves.

Optical density and total kjeldahl-nitrogen of heat clarified sap of infected plants increased with the increasing concentration of potassium upto 390 ppm. Beyond this, there was a decrease both in optical density and total kjeldahl-nitrogen in diseased plants (Table V).

TABLE V.

Effect of potassium and virus infection on optical density and kjeldahl-nitrogen (total) of sap of diseased plants of Turkish tobacco (N. tabacum *L.*)

Potassium	Interval (days)*				
	20	35		50	
		Optical density	Kjeldahl-nitrogen mg./ml.	Optical density	Kjeldahl-nitrogen mg./ml.
0 ppm	—	0·272	0·476	0·328	0·476
39 ppm	—	0·350	1·40	0·555	1·19
390 ppm	—	0·390	1·681	0·530	1·09
3900 ppm	—	0·342	1·232	0·490	0·910

* Time between transplantation and harvest of plants. Optical density readings were taken at 265 mμ wave length and 1 cm. light path using Unichem spectrophotometer.

Discussion

Bawden and Kassanis (1950) conducted experiments with *N. tabacum* L. var. white burley, raised in a mixture of infertile soil, sand and peat and supplied with K_2SO_4. They suggested that potassium slightly reduced the TMV concentration of plant sap though it usually increased plant size and total virus content per plant. Cheo *et al.* (1952), on the other hand, showed that the growth (fresh weight) of *Spinacia oleracea* L. (spinach) plants, grown in washed quartz sand, was the highest at 430 ppm of potassium and there was pronounced stunting above and below this level. Rate of formation of cucumber virus 1 in spinach plants coincided with its growth pattern. Pound and Weathers (1953) reported that growth (fresh weight) of *N. glutinosa* L. and *N. multivelvis Lindl.*, grown in washed sand, was maximum at 78 and 704 ppm of potassium respectively. The concentration of turnip virus 1 in these plants was also highest under these conditions.

Our results show that the severity of the virus symptoms followed closely the growth of the plants. Concentration of TMV in plant sap was not proportional to the growth. Plants supplied with 39 and 390 ppm of potassium, although differed in growth, produced almost the same concentration of virus. Similar was the case with optical density and total kjeldahl-nitrogen. Our findings, within the limits, agree with those of Bawden and Kassanis (1950) and Cheo *et al.* (1952).

In our experiments very little virus was formed during the 30 days at 0 ppm of potassium and it was highest at 390 ppm. Beyond this the virus formation was inhibited. In this respect potassium does not behave like phosphorus and nitrogen which are known

to promote virus protein synthesis under the conditions of high nutrient supply, even though growth is adversely affected (Bawden and Kassanis, 1950; Selman and Grant, 1957; Spencer, 1939; 1941).

Effect of potassium nutrition on plant metabolism has been reviewed by Thomas *et al* (1955). Potassium is a mobile element which accumulates easily in leaves and the meristematic cells; vegetative developments are favoured by abundant supply. Further, potassium nutrition slightly enhances the water content per unit area of leaf. It is probable that virus activity is affected by differences in potassium supply, by upsetting one or all of these vital metabolic processes.

Summary

The effect of different levels of potassium, on height, fresh and dry weights of healthy and tobacco mosaic virus infected Turkish tobacco (*Nicotiana tabacum* L.) plants was studied. When the concentration of potassium was increased or decreased beyond 390 ppm, adverse effect, both in infected and control plants, was observed. The fresh and dry weights of infected plants fell considerably below normal but the height was not much affected. Increase of potassium beyond 390 ppm resulted in slightly decreased virus production in plants. Optical density and total kjeldahl-nitrogen of heat-clarified sap of infected plants increased with the increasing concentration of potassium upto 390 ppm after which there was a decrease in both.

Acknowledgment

The authors are grateful to Dr. B. M. Gupta, Central Drug Research Institute, Lucknow for ungrudging help during the course of the experiments. Our thanks are also due to Dr. P. S. Krishnan, Professor of Biochemistry, Lucknow University for permission to use Unichem spectrophotometer. The authors wish to record their thanks to the Scientific Research Committee U.P. for financial help.

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Explanation of Plates

Plate I

FIGURE 1.

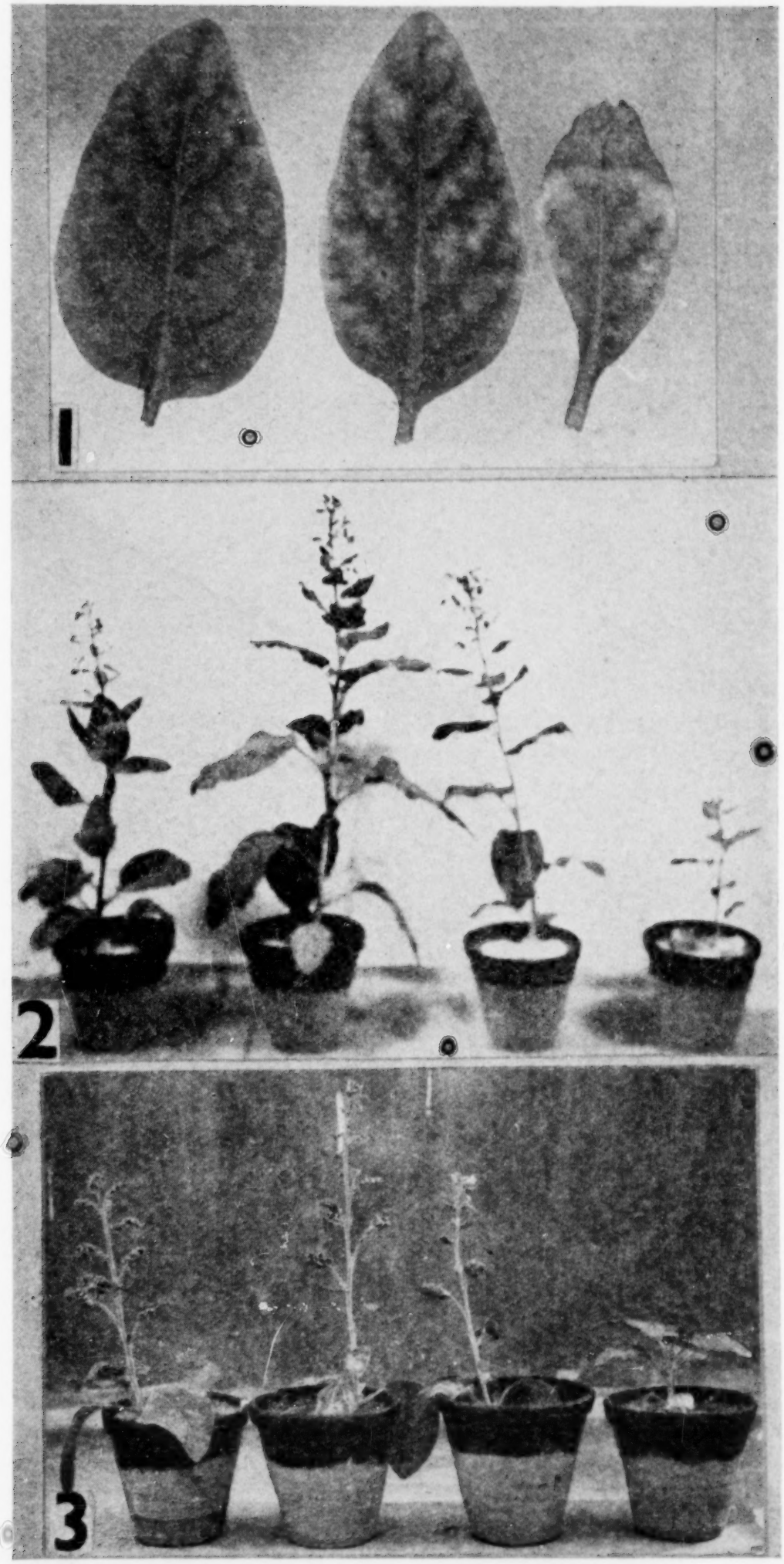
Potassium deficiency symptoms in leaves of Turkish tobacco plants. There is interveinal chlorosis and scorching of tips and margins.

FIGURE 2.

Turkish tobacco plants, 50 days after transplantation, from left to right showing the effect of 3900, 390, 39 and 0 ppm of potassium.

FIGURE 3.

Effect of potassium on TMV infected Turkish tobacco plants. Plants, 50 days after transplantation from left to right receiving 3900, 390, 39 and 0 ppm of potassium.



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It is seen from Table III that following the usual practice of centrifuging at 3,000 r.p.m. and discarding the supernatant fluid, viable tubercle bacilli are discarded which can produce approximately 24% more colonies of tubercle bacilli. This is a considerable loss and we therefore subjected sputum, which previous examination had shown to have only very few or no demonstrable A.F.B., to homogenisation with 4% NaOH for 10 min. and centrifugation at 18,000 r.p.m. and the usual washing with sterile distilled water. As most of the samples received in this laboratory for culture of T.B., have been first screened by repeated microscopic examination by clinics and found to be negative, centrifugation at 18,000 r.p.m. is now a routine procedure.

The effect of good liquefaction and centrifugation is also illustrated in the following table which gives results obtained by centrifuging for 5 min. homogenised sputum at varying centrifugal speeds—namely (1) in a hand centrifuge, (2) in an electrical centrifuge at 3,000 r.p.m. and (3) in a refrigerated centrifuge at 18,000 r.p.m. It was considered important that trials should be conducted after spinning in a hand centrifuge because the method of concentration with 1% sodium carbonate is recommended for small clinics where a hand centrifuge is often the only centrifuge available. Centrifuging for 5 min. was selected because it was difficult to manipulate a hand centrifuge at a reasonably good and uniform speed for more than 5 min.

It will be observed from Table IV.

(1) that the carbonate method gives better results than Hank's flocculation method after centrifuging by all the three methods adopted and that the difference in positive findings is most marked in the results obtained by spinning in a hand centrifuge. This was considered to be due to the better liquefaction of the sputum sample by 1% $NaCO_3$ than that obtained by Hank's method.

(2) that the percentage of positive findings increased with increased centrifugal speeds in that it rose from 12% obtained by hand centrifuging to 88% with very high-speed centrifuging.

(3) that the difference of positive findings between the two methods of concentration disappear when adequate centrifuging is possible.

Summary

Concentration of sputum using 1% sodium carbonate is recommended as the method of choice for those laboratories depending upon microscopic examination for the bacteriological diagnosis of tuberculosis.

When culture of tubercle bacilli is intended, especially for specimens containing few or no demonstrable tubercle bacilli, centrifugation in a refrigerated centrifuge at 18,000 r.p.m. after homogenising with 4% sodium hydroxide is recommended.

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INDIAN
JOURNAL OF
MICROBIOLOGY

Vol. I, No. 1, 1961

SURVEY OF *CANDIDA SPP.* IN EIGHT HUNDRED CASES OF LEUCORRHOEA

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(Received for publication, November 1959)

Recently, mycotic infections have been found to be increasing at an alarming rate (Lee and Keifer, 1954). This is thought to be due to the use of broad-spectrum antibiotic-therapy (Bratland and Hotten, 1954; Brown *et al.*, 1953; Brown, 1954; Chaplan, 1955; Huppert and Cazin, 1955; Marconi, 1956; Sharp, 1954; Woods *et al.*, 1951). The present communication deals with the survey of *Candida spp.* in 800 cases of leucorrhoea.

MATERIALS AND METHODS

Vaginal discharge of patients with leucorrhoea attending the outpatients-departments of the Hospitals of Chittaranjan Seva Sadan College of Obstetrics, Gynaecology and Child Health, were examined after smear-preparation and staining by Gram's method. Those cases which showed the presence of yeast-like fungi in the smear-examination and the development of colonies in Nickerson's medium were selected for further mycological studies. The morphological characteristics were also studied after staining by Mcguire's technique as given by Dey (1958). For the study of cultural characteristics, the yeast-like fungi were grown in Nickerson's and Sabouraud's dextrose-agar media. Fermentation reactions were studied in glucose, maltose, sucrose and lactose sugar media. Chlamydospore formations were observed in human serum as a culture medium. The full description of this technique will be published elsewhere.

Albino mice (18-20 g.) were injected subcutaneously in the dorsum of the neck with saline suspension (0.25 ml./animal) of *Candida spp.* from Sabouraud's dextrose-agar media. There were 1-2 million organisms/ml. Smears from the site of injection were taken daily after making a nick with scalpel and were studied after staining. In all, 60 albino mice were used, 15 for each of the species, *C. albicans*, *C. tropicalis*, *C. krusei* and *C. stellatoidea*.

In rabbits, the injections were given per vein. The dose was 0.5 ml./animal. Four rabbits were used for *C. albicans*, 2 for *C. tropicalis*, 2 for *C. krusei* and 3 for *C. stellatoidea*.

In order to study the intercommunicability of the yeast-like fungi, the mouth of babies from mothers with vaginal candidiasis and the external genitalia of husbands were examined.

Results

Eight hundred cases of leucorrhoea were screened for infection by yeast-like fungi. Twenty-five per cent of these cases showed *Candida spp.* infection. *C. albicans* was found in 51%, *C. krusei* in 5%, *C. tropicalis* in 29%, *C. stellatoidea* in 13.5%, *C. pseudotropicalis* in 1% and *C. quillfermondi* in 0.5% of the cases. Gram-positive yeast-like fungi were detected in the vaginal smears of 178 patients. They existed in the form of spores, or well-developed mycelial filaments with typical septa and blastopores characteristic of *Candida spp.* In Nickerson's medium, from 200 cases, characteristic black or deep-brown colonies developed, proving the importance of taking cultures even during the preliminary investigation.

C. albicans in Nickerson's medium produced a peculiar type of colonies. They were black or deep brown in colour, size resembling a pin-head with spider-like process. These processes produced branching and sub-branching resembling roots of plants (Fig. 1). *C. tropicalis*, *C. krusei* and *C. stellatoidea* produced black or deep brown colonies, size varying from pin-head to pin-point but without radiating processes. The growth usually appeared within 72-96 hr. (Figs. 2, 3, 4).

All species of *Candida* produced creamy-white to pale-white colonies on Sabouraud's dextrose-agar medium.

In experiments with mice, *C. albicans* produced mycelia with spores and budding spores in the subcutaneous tissue (Fig. 5). Local abscess formation was also observed at the site of injection after 48 hr. On the 6th day, the skin over the site of injection sloughed out spontaneously, leaving an excavating ulcer (Fig. 6). On the 7th day, smears showed pus cells with spores and mycelia. The spores and mycelia diminished in size and number after the 7th day and completely disappeared by the 15th day, when only a few pus cells were found. Cultures were positive up to the 10th day. The ulcers were found to heal by the end of four weeks.

In *C. tropicalis*, *C. krusei* and *C. stellatoidea*, there were gradual development of spores and mycelia up to the 4th day after injection, but there was no ulcer-formation.

Rabbits were found to die within 4th to 6th day after injection with *C. albicans* and after 2 to 3 days with *C. stellatoidea*. In cases of *C. tropicalis* and *C. krusei*, none of the animals died.

Intercommunicability seems to be the characteristic feature of *C. albicans* and of no other species of *Candida*. The husbands (800) of the patients were screened; 15 had balanoposthitis due to *C. albicans*. Babies of patients showed mouth-infection of *C. albicans*. Out of 55 obstetric patients screened, 5 showed the development of thrush due to *C. albicans*.

Summary

Out of eight hundred cases of leucorrhoea screened for *Candida spp.* infection 25% were found positive. Culture of vaginal swab was observed to be the best method

for diagnosing *Candida* infection. *C. albicans* and *C. stellatòidea* were found pathogenic to mice and rabbits. *C. albicans* seems to be intercommunicable in human beings.

ACKNOWLEDGMENT

Our thanks are due to Dr. Subodh Mitra, Director and Principal of Chittaranjan Seva Sadan College of Obstetrics, Gynaecology and Child Health and Chittaranjan Cancer Hospital for his keen interest in this work. Our thanks are also due to Messrs. Sarabhai Chemicals, under whose grant-in-aid this work was carried out.

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EXPLANATION OF PLATES

FIG. 1. Growth characteristics of *Candida albicans*: 192 hr. growth in Nickerson's medium.

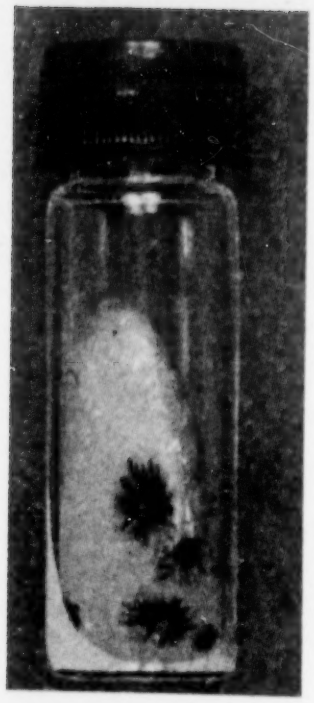
FIG. 2. Growth characteristics of *C. tropicalis*: 192 hr. growth in Nickerson's medium.

FIG. 3. Growth characteristics of *C. krusei*: 192 hr. growth in Nickerson's medium.

FIG. 4. Growth characteristics of *C. Stellatoidea*: 192 hr. growth in Nickerson's medium.

FIG. 5. Growth characteristics of *C. albicans*, *C. tropicalis* and *C. krusei*: 24 hr. growth in Sabouraud's dextrose-agar medium.

FIG. 6. Development of spores and mycelia in the subcutaneous tissue of mice.



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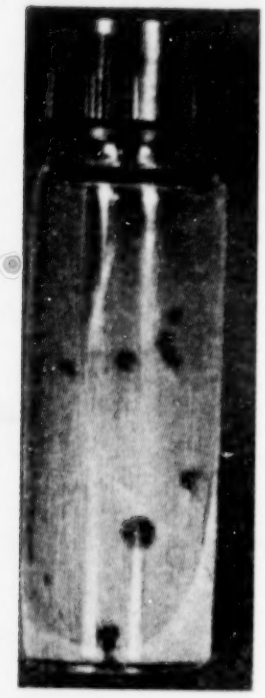

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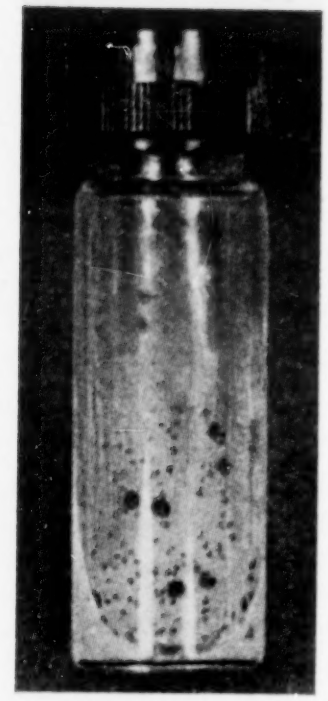

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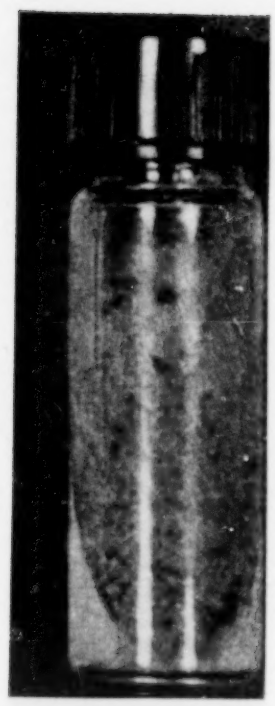

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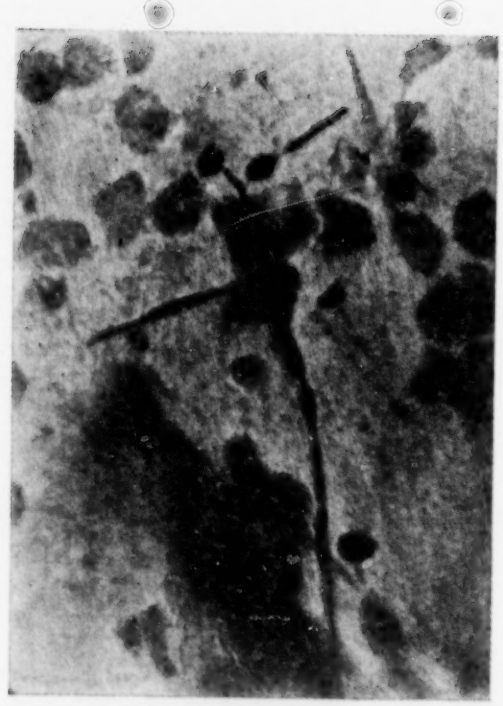

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Two copies of each manuscript should be submitted. A short running title, suitable for page-headings, should be furnished. The name of the laboratory where the work was done should be indicated on the title page.

Authors are responsible for preparing a paper in a form suitable for sending to press. Careful preparation of manuscript will make for prompt publication.

Illustrations may, if possible, be drawn on Bristol board in Indian ink with lettering inserted lightly in pencil. Author's name, short title of the paper, fig. no. etc. should be marked at the back of the illustration. Drawings may be larger than the size of the printed block, and their order and approximate position in the text should be marked. Line drawings will be referred to as Figure 1, Figure 2, etc and half-tone blocks as Plate I, Plate II, etc. Besides the original illustrations one duplicate set must accompany the second copy of the manuscript.

Tabular matter may be kept to a minimum.

Spelling should conform to current English usage according to the Concise Oxford Dictionary.

Binomial Latin names of micro-organisms should be given in full when first mentioned in a paper and subsequently with the generic name abbreviated. They should be underlined in the typescript.

The following symbols and abbreviations may be written in the manner shown: degrees Centigrade are written, *e.g.* 100°; hr., min., sec. (singular and plural); *M*, Molar; *N*, normal (of solutions); m, milli-(10^{-3}) and, micro-(10^{-6}); *e.g.*, ml., millilitre (instead of cc.) and μ g. (instead of γ), microgram; No. or no., number; dilutions should be written 1/10.

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References to books and monographs should include the town of publication and the name of the edition to which reference is made. The arrangement should be as in the following example: Kudo, R. R. (1954), *Protozoology*, 4th edition, Charles C. Thomas, Springfield, Illinois, U.S.A.

Citations in the text should read thus: (Bose, Ghose and Roy, 1950). When a paper has more than two authors, the names of all of them should be given at its first citation in the text (unless there are more than five authors) and in subsequent citations thus: (Bose *et al.*, 1959). The conventions Bose, (1959a), Bose, (1959b) should be used where more than one paper by the same authors has appeared in one year.

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this also is the first authentic record of this disease in India. In some of the farms, Goshalas and clinics, examination of the vulva and vagina of different species of animals revealed a very high incidence of vulvovaginitis to the tune of 82·9 to 100·0% in cattle, 46·4 to 66·6% in buffaloes, 22·7 to 33·3% in goats and 100·0% in sheep. The disease occurred in animals of all age groups, except in very young animals and in majority of cases examined, the disease was found to occur in a very severe form. A purulent discharge, probably associated with pyaemic infection, was seen in several animals suffering from vulvovaginitis.

The gross pathological lesions seen in the vulva and vagina of affected animals have been described and their significance in the diagnosis of the disease has been discussed.

The diagnosis of its virus origin was based on negative bacteriological findings.

SECTION B. PLANT VIRUS

37. TRENDS IN RESEARCH ON PLANT VIRUSES AND VIRUS DISEASES IN INDIA ; R. S. VASUDEVA, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

Viruses have come to occupy a very important place in the human economy due to the diseases they cause in man, domesticated and wild animals, insects, plants and even bacteria. They have been a scourge of mankind since before the dawn of recorded history. Small pox, for example, existed in China in 1700 B.C. Measles, mumps, influenza and scarlet fever are some of the other virus diseases that affect human beings. Next in importance are the virus diseases of plants which indirectly affect the common man by robbing him of his due share of food.

The virus diseasès are responsible for serious losses to our agricultural crops. They are all the more important in the plantation crops as also those which are propagated vegetatively. Ever since the discovery of tobacco mosaic virus in 1892, over 300 different viruses attacking plants have been described. Most of the economic crops are affected with one or the other virus. Although accurate figures of losses due to these are not available it is now well known that the damage they cause probably equals that due to all other disease-causing agents. Due to the serious nature of these diseases, plant virus diseases have received considerable attention in other countries and the science of Virology has made considerable progress. The most outstanding advances made are the isolation and crystallization of several viruses, studies of their chemical composition, biochemical studies and the use of serological methods in the identification and differentiation of viruses and virus strains.

The science of Virology is comparatively of recent origin in India. Although the spike disease of sandal was reported to be graft transmissible by Coleman as early as 1917 systematic research on plant viruses in India started only about a quarter of a century ago. During this period several diseases of economic crop plants, fruits and vegetables as well as plantation crops have been investigated in order to evolve suitable methods of their control. Among the earliest described diseases is the tobacco leaf curl, a virus that is transmitted in nature by the white fly (*Bemisia tabaci* Gen.) and has a very wide host range which includes tomato, papaya, chilli, Sann-hemp and a large number of

weeds and ornamental plants. The potato crop has been reported to be affected by several viruses in India such as potato virus X, Y, A, D, and leaf roll which are responsible for heavy losses in yield and degeneration of seed stocks. Sugarcane mosaic 'little leaf' of brinjal and yellow vein mosaic of *Bhindi* are some other diseases that were described in early days.

A comparatively rapid progress has been made during the last fifteen years and a large number of virus diseases such as mosaic of Bottlegourd, Sann-hemp, papaya, cardamon, lettuce, cowpea and soyabean, 'grassy shoot' of sugarcane, mosaic and bunchy top of banana, 'Foorkey' and 'Chirke' diseases of large cardamon, 'small leaf' of cotton, sesamum phyllody, coconut wilt and citrus decline have been described and their vectors reported. The study of virus diseases of stone fruits have also received attention and 'line pattern' of plum, plum mosaic, marble mosaic of cherry, mosaic of *Rubus ellipticus*, peach mosaic, variegated mosaic of apple and 'line pattern' of almond are some of the diseases recorded so far.

Although most of the earlier work on plant viruses in India relates to the study of the symptomatology and host range, determination of vectors and identification of the viruses, during the recent years attempts are being made to tackle the problems of fundamental nature. Virus-vector relationship studies have been conducted with respect to a number of virus diseases. Studies on the purification of plant viruses and their bio-physical and biochemical properties have been initiated. The shape and size of the virus particles is being studied. The importance of serological studies on plant viruses which had received little attention in the past has now been recognised. It has now developed into an important branch of virus study and is a valuable tool in differentiation and identification of viruses and virus strains.

As the knowledge about plant viruses is rapidly increasing, new techniques or procedures are being devised to replace the existing methods of control. While the seed certification and the use of virus-free seed potatoes is receiving wider application and resistant or tolerant varieties have been determined for yellow vein mosaic of *Bhindi*, chilli mosaic, 'small leaf' disease of cotton and papaya mosaic, the possibilities of use of heat and chemotherapeutic treatments in the control of plant virus diseases are being explored. In this respect studies on the inhibition of viruses by various chemicals, anti-biotics and other agents have been initiated. The recent introduction of systemic insecti-cides has opened new opportunities for the direct control of insect vectors and a number of systemic insecticides such as Ekatox and Folidol have been included in the trials for control of tomato leaf curl and other virus diseases at the Indian Agricultural Research Institute.

The control of plant viruses by inoculating plants with mild or attenuated strains of viruses with a view to protecting against infection with severe ones is another measure which holds promise in the future. Although in this method of control there are chances of contaminating the crop besides having the danger of synergistic effects, of chance infection with other viruses, the method needs to be given a fair trial as it would perhaps be a lesser evil.

Another useful line of control which has been mostly neglected till today is the biological control of plant viruses. It is well known that several fungi and viruses parasitise the insects and this fact could be exploited in the interests of the agriculturist. It would,

this also is the first authentic record of this disease in India. In some of the farms, Goshalas and clinics, examination of the vulva and vagina of different species of animals revealed a very high incidence of vulvovaginitis to the tune of 82·9 to 100·0% in cattle, 46·4 to 66·6% in buffaloes, 22·7 to 33·3% in goats and 100·0% in sheep. The disease occurred in animals of all age groups, except in very young animals and in majority of cases examined, the disease was found to occur in a very severe form. A purulent discharge, probably associated with pyaemic infection, was seen in several animals suffering from vulvovaginitis.

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Another useful line of control which has been mostly neglected till today is the biological control of plant viruses. It is well known that several fungi and viruses parasitise the insects and this fact could be exploited in the interests of the agriculturist. It would,

therefore, be of interest to study the fungal and virus diseases of insects particularly the vectors with a view to explore the possibilities of biological control.

The research on plant viruses in India has so far been chiefly confined to the Indian Agricultural Research Institute and a few other central Agricultural Institutions in the country. It is high time that these studies are taken up at University level and by the States. A beginning has already been made in this direction and it is hoped that adequate facilities will be provided in the State Institutes as also Universities. What is required is a team work by plant pathologists, biochemists, physicists, plant physiologists and geneticists to solve some of our more intricate problems e.g., root diseases of coconuts which are widespread in coconut-growing countries of the world and have almost shattered their economy.

38. MULTIPLICATION OF COLIPHAGE (CVX-5) IN *ESCHERICHIA COLI* ; B. M. GUPTA, CENTRAL DRUG RESEARCH INSTITUTE, LUCKNOW

Coliphage (CVX-5) was isolated from bacteria-free filtrate of a faeces of a patient of colitis. It produced big and small plaques when plated on *Escherichia coli* (Kasauli strain) but only small plaques on *Shigella shigae* and *Eberthella typhi*. *Vibrio cholerae* and *Staphylococcus aureus* were not lysed. The phage was purified by single plaque technique and differential centrifugation.

The main features of plaque formation by CVX-5 on *E. coli*—the time of first appearance of plaque, the rate of plaque formation, period of peak production and 50% yield time—were studied. The lag period was estimated between 135 and 165 min., the period of peak production between 195 and 225 min. and 50% yield between 202 and 316 min. The rate of plaque formation works out to 21-35% per unit increase in log time. Most of the phage-resistant strains isolated were purine-requiring. Adenylic acid (adenosine-3-phosphate) increased the plaque count and plaque size considerably, when it was incorporated into the host agar plate prior to incubation.

39. A VEIN-BANDING MOSAIC DISEASE OF CHILLIES (*CAPSICUM FRUTESCENS* L.) ; K. S. BHARGAVA AND R. D. JOSHI, DEPARTMENT OF BOTANY, GORAKHPUR UNIVERSITY, GORAKHPUR, U.P.

A vein-banding mosaic disease of chillies (*Capsicum frutescens* L.) found prevalent in fields near Bhowali has been studied. The causal virus is mechanically transmitted to different commercial varieties of chillies, and also to other solanaceous plants. *Myzus persicae* Sulz. and *Aphis gossypii* Glov. can transmit the disease after short infection-feeding. It is not transmitted through seeds of chillies. It resembles potato virus Y in physical properties and host-range, but differs from it in its reaction towards tobacco in which it induces necrosis of veins, petiole and stem. Serologically it is related to potato virus Y, but both do not cross-immunize in tobacco plant. It is, therefore, regarded as a variant strain of potato virus Y similar to tobacco veinal necrosis virus.

40. INSECT TRANSMISSION OF CHILLI MOSAIC DISEASE; T. K. NARIANI AND K. S. M. SASTRY, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

It has been found that a single viruliferous aphid (*Aphis gossypii* Glove) is capable of transmitting the virus to healthy plants. The aphids could transmit the virus after feeding on infected plants for 30 sec. but the maximum infection was obtained when feeding period was 5 min. The infectivity of the viruliferous aphids decreased with longer feeding intervals. Previous fasting of the aphids increased their efficiency in transmitting the virus in the case of short acquisition feeding.

41. POTASSIUM NUTRITION OF TURKISH TOBACCO (*NICOTIANA TABACUM* L.) PLANTS IN RELATION TO MULTIPLICATION OF TOBACCO MOSAIC VIRUS; G. S. VERMA AND J. P. VARMA, DEPARTMENT OF BOTANY, LUCKNOW UNIVERSITY, LUCKNOW

Turkish tobacco (*N. tabacum* L.) was used as host plant. Arnon and Hoagland's nutrient solution with 0, 39, 225 and 390 ppm concentrations of potassium was utilised as nutrient solution. In each set of experiments, when potassium deficiency symptoms of intervienal chlorosis and stunted growth of plants was evidenced, half the number of plants were inoculated with tobacco mosaic virus which in course of a week exhibited systemic disease symptoms and an equal number of plants were inoculated with sterile distilled water (controls). In both healthy and diseased plants fresh weight increased regularly with increasing concentrations of potassium, being heighest at 390 ppm while height was relatively less affected. Leaf-stem ratio remained irregular in relation to differential potassium nutrition, although it increased in diseased plants. Virus concentration in leaf and stem juice when estimated by counting the local lesions produced on half leaves of test plant (*Nicotiana glutinosa* L.) and weighing the dried virus protein did not show the same relation as exhibited by fresh weight, height or leaf stem ratio. Supplements of 225 and 390 ppm to potassium deficient plants enhanced the fresh weight but failed to increase the virus concentration. The results indicated that variations in potassium supply had marked effects on host growth but not on virus multiplication except when very high amounts of this nutrient were supplied. Total nitrogen and total soluble protein contents were consistently greater in leaf juice of diseased than of their healthy counterparts following the pattern of virus concentration.

42. NITROGEN NUTRITION OF TURKISH TOBACCO (*NICOTIANA TABACUM* L.) IN RELATION TO MULTIPLICATION OF TOBACCO MOSAIC VIRUS; J. P. VARMA, DEPARTMENT OF BOTANY, LUCKNOW UNIVERSITY, LUCKNOW

Height of stem and fresh weight per plant of Turkish tobacco (*Nicotiana tabacum* L.) grown in purified sand were studied by supplying zero, low, medium and high amounts of nitrogen in nutrient solution. Typical deficiency symptoms remained prominent in plants receiving zero and low amounts of the nutrient. Host plant response to various concentrations of nitrogen in nutrition was also usually similar even after tobacco

mosaic virus infection. Virus concentration, estimated by local lesion count method in expressed saps from leaf tissue samples, regularly increased with increasing amounts of nitrogen in nutrient solution supplied to the host plant. The direct relationship of tobacco mosaic virus with amounts of nitrogen was so consistent that even on withholding nitrogen supply to plants previously grown at higher levels, or on supplementing it to deficient ones, it existed almost parallel to available nitrogen irrespective of the height, fresh weight and leaf-stem ratio of the host plant.

43. MAGNESIUM NUTRITION OF TOBACCO AND PETUNIA PLANTS IN RELATION TO MULTIPLICATION OF TOBACCO MOSAIC VIRUS; J. P. VARMA, DEPARTMENT OF BOTANY, LUCKNOW UNIVERSITY, LUCKNOW

Turkish tobacco (*Nicotiana tabacum* L.), *N. glutinosa* L. and Petunia (*Petunia* sp. L.) used as host plants, were grown in sand cultures watered with Arnon & Hogland's nutrient solutions containing 3 different levels *viz.* 0, 48, and 480 ppm of magnesium. When magnesium deficiency symptoms of uniform yellowing appeared on very small leaves and stunted growth of plants became evident, half the number of host plants were inoculated with tobacco mosaic virus, rest were left uninoculated (controls). It was found that magnesium affected the height and fresh weights of both inoculated and uninoculated host plants; height and weight were maximum at 48 ppm, leaf-stem ratio being irregular.

With the increase of magnesium nutrition of host plant the virus concentration, measured by spectrophotometric and local lesion count method, showed a simultaneous increase. The height, fresh weight and leaf-stem ratio in the plant did not correspond to or show any relationship with virus multiplication. This relation of magnesium with tobacco mosaic virus concentration was consistently exhibited when amounts of this nutrient in nutrition were altered variously during the course of experiments.

44. YELLOW-NET VIRUS DISEASE OF TOBACCO PLANT; K. L. DHINGRA AND T. K. NARIANI, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

A disease of tobacco plant, characterised by severe veinal chlorosis, yellow network of veins and veinlets, was found to be caused by a virus. The virus could be transmitted from diseased to healthy plants by grafting and also by white fly (*Bemisia tabaci* Gen). It also attacked *Beta vulgaris* and aster plant. Cross protection tests showed that the yellow-net virus is related to tobacco leaf curl virus. It appears to be a new virus.

45. ANTIGENICITY OF PURIFIED BOTTLE GOURD MOSAIC VIRUS; G. P. ANAND AND M. D. MISHRA, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

Purified preparation of the virus was intravenously injected in albino rabbits at weekly intervals. The antiserum obtained from the immunised rabbit was found to give

a clear flocculent precipitate with the sap of diseased plants but not with the sap of healthy plants. The optimum precipitation occurred at 1 : 2 dilution of antiserum and 1 : 4 dilution of the virus.

SECTION C. BACTERIAL VIRUS

46. PHAGE TYPING OF *ESCHERICHIA COLI* OF ANIMAL AND HUMAN ORIGIN ; S. S. KASATIYA AND C. M. SINGH, DEPARTMENT OF PATHOLOGY, VETERINARY COLLEGE, MATHURA, U.P.

Studies were conducted on the phage pattern of 138 strains of *Escherichia coli* from animals and 62 strains from human sources including strains associated with infantile diarrhoea from Christian Medical College & Hospital, at Vellore and strains isolated from cases of appendicites from Creighten Freeman Christian Hospital, Vrindaban. Forty three *E. coli* phages were isolated locally from faecal samples of man and animals and were compared with 21 foreign phages including 1 human phage obtained from Central Drug Research Institute, Lucknow, 13 animal phages from Dr. Smith of Animal Health Trust Essex, England, and 7 human phages from Dr. Nicolle of Pasteur Institute, Paris. Isolation of *E. coli* phages from faecal samples and sewage was found more dependable than Fisk's cross-culture technique. For propagation of phages heart extract broth was found superior when compared with nutrient broth and Robinson's medium.

Out of 200 strains, 59 did not show lysis by the group of 21 foreign phages. Hence 43 phages were isolated locally for typing these strains. The remaining 141 strains of *E. coli* from man and animals were grouped into 42 phage types, 12 strains being still ungroupable. Phages isolated from animal origin were seen for lysis over human *E. coli* strains and vice versa. The study gave an indication that the phage pattern of the strains of animal origin may be different from that of the strains of human origin. An observation was made to relate the maltose fermentation in the serological group of *E. coli* 055 : B5 to the prevalent phage type. Phage types present in 21 strains of this serological group in which maltose was fermented within 24 hr. were more or less similar but different from those of the strains which fermented maltose later.

While observing a difference in phage lysis between the strains isolated from normal animals and diseased, it was seen that the strains isolated from pathological conditions were not easily lysed or if at all they were the number of phages acting on them had been only one or two. Of the 67 strains of *E. coli* isolated from diseased conditions in man and animals 80% of the strains were lysed by only one or two of 64 phages in total and 20% did not show any lysis.

Fifteen strains of *E. coli* 055 : B5 obtained from cases of infantile gastro-enteritis on which 43 phages locally isolated did not show any lysis were sent to Dr. Nicolle for further phage typing. Out of this lot 9 were found to belong to St. Christopher phage type and the remaining 6 perhaps belonged to some other new type.

According to Dr. Nicolle the existence of St. Christopher phage type in India is very interesting as this is common in Great Britain, but very rare in the continent, in Germany and Hungary. Some strains have been found in Indo-china.